Motor-Dependent and -Independent Roles of CENP-E at Kinetochores: The Cautionary Tale of UA62784

Centromere Protein E (CENP-E) is a kinetochore-associated motor protein of the kinesin-7 family. During mitosis, cells use the ATPase activity of CENP-E to slide misaligned chromosomes along spindle microtubules toward the equator and establish the metaphase plate prior to chromosome segregation. In addition to its requirement as a molecular motor for chromosome motion, we have recently reported a motor-independent role of CENP-E in the recruitment of CLIP-associated proteins (CLASPs) to kinetochores, thereby promoting turnover and poleward flux of attached microtubules (Maffini et al., 2009). This conclusion was drawn in part after the use of a small molecule called UA62784 that had been isolated from a high-throughput cytotoxicity screen against pancreatic cancer cells and was reported to be a specific inhibitor of the ATPase activity of CENP-E (Henderson et al., 2009).

In a recent article in *Chemistry & Biology*, Tcherniuk et al. (2011) reinvestigated the use of UA62784 as a CENP-E inhibitor and reported that differing from the original observation, UA62784 does not inhibit CENP-E ATPase activity in vitro. Instead, they found UA62784 to be a potent inhibitor of microtubule polymerization and highly cytotoxic at nanomolar concentrations. This important study, while clarifying the cellular target of UA62784, challenges the conclusions previously drawn from the use of this small molecule in basic research studies addressing CENP-E function.

Here, we would like to argue that despite the inadvertent use of UA62784 as a published CENP-E inhibitor, our previous conclusions regarding a motor-independent role of CENP-E in recruiting CLASPs to kinetochores remain valid based on the following lines of evidence. First, CENP-E depletion by RNAi in HeLa cells prevented normal CLASPs recruitment to kinetochores specifically, but not to centrosomes and spindles. Second, mitotic cells (blocked with micromolar doses of nocodazole) did not show any alteration in CLASPs recruitment to kinetochores upon treatment with 100 nM UA62784, a concentration now reported to completely disassemble the mitotic spindle in HeLa cells (Tcherniuk et al., 2011). Importantly, in our study, the conclusions we drew from the use of UA62784 were supported with experiments involving overexpression of a motor-less CENP-E construct (GFP-CENP-E NΔ803), which causes a dominant-negative effect by replacing endogenous CENP-E at kinetochores (Schaar et al., 1997). Under these conditions, CLASPs recruitment to kinetochores remained unaltered. Finally, treatment of mitotic HeLa cells with the recently developed CENP-E allosteric inhibitor GSK-923295 (Wood et al., 2010), currently in phase I human clinical trials, did not cause any perturbation in CLASPs recruitment to kinetochores (Figure 1).

Taken together, this set of self-contained data substantially supports a motor-independent role of CENP-E in

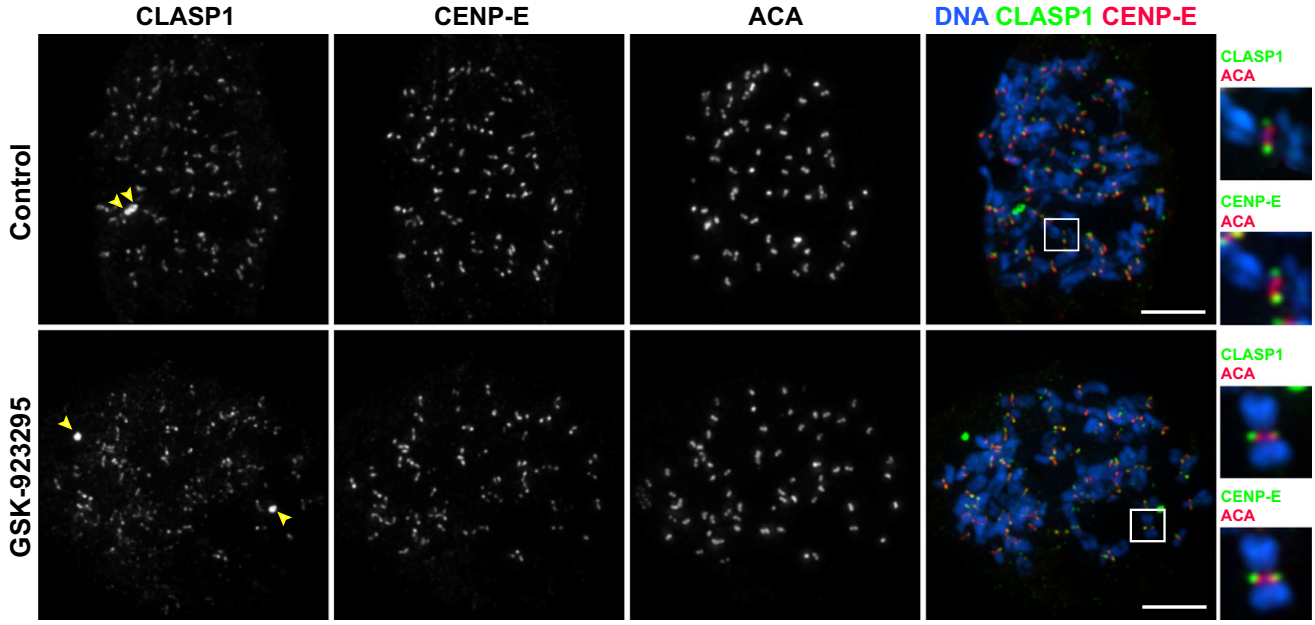

Figure 1. CLASP1 Localization at Kinetochores Is Independent of CENP-E Motor Activity
HeLa S3 cells were processed for chromosome spreads following incubation for 1.5 hr with 2 μM nocodazole in the absence (Control) or in the presence of 20 nM CENP-E inhibitor (GSK-923295; MedChemexpress Co., Ltd.), a concentration that we determined to be sufficient to cause a penetrant CENP-E phenotype with few misaligned chromosomes in most mitotic cells. Chromosomes were subsequently immunostained for CLASP1, CENP-E, and anti-centromere antigens (ACA); for details about antibodies and methods, see Maffini et al., 2009. DNA was counterstained with DAPI. Insets are 3× magnifications of the indicated chromosomes. Arrowheads indicate centrosomes. Scale bars represent 5 μm.

recruiting CLASPs to kinetochores in human cells, in agreement with our previous conclusions (Maffini et al., 2009). In this regard, the work of Tcherniuk and colleagues succeeded in raising a word of caution for the inadvertent use of UA62784 as a CENP-E inhibitor as well as in challenging conclusions based on the sole use of small molecule-inhibitors in general, when not accompanied by complementary experiments. It is thus opportune to say that drugs can not only kill you, but also kill your reputation. Fortunately, drugs can also save you!

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